

Original Research

# Analysis of Calcaneal Bone Mineral Density (cBMD) in Healthy College Students

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### Background

There is limited evidence describing the relationship between calcaneal bone mineral density (cBMD) and activity level, menstrual history, or the development of bone stress injury (BSI).

### Hypothesis/Purpose

The purposes of this study were to: 1) examine the influence of physical activity on cBMD in healthy college students (HCS), 2) determine if there is an association between cBMD, body mass index (BMI), sex, menstrual history, and history of BSI in HCS, and 3) compare the cBMD of HCS to cBMD data collected on intercollegiate athletes (ICA) from a previous study.

### Study Design

Cross-sectional design

### Methods

This cross-sectional study recruited a convenience sample of HCS at one institution. Subjects provided self-reported injury and menstrual history, completed a physical activity questionnaire, and cBMD and BMI measures were obtained. Descriptive statistics, statistical analyses of relationships (Chi-square and relative risk), logistic regression, and differences (t-tests) were used in the statistical analyses.

### Results

One hundred three HCS (82 female, 21 male; age  $21.9 \pm 1.13$ ) consented to participate. The composite score for work, leisure, and sport activity ranged from 5.6 to 11.1 ( $7.9 \pm 1.1$ ) for HCS subjects. There was no significant correlation between cBMD and physical activity in HCS, however, a significant correlation was found between reported age of onset of menstruation and left and right cBMD ( $r = -0.22$  and  $r = -0.23$ ;  $p < 0.05$ ) and history of secondary amenorrhea and history of BSI ( $r = 0.32$ ;  $p < 0.05$ ). There was no difference in cBMD between the male ICA and male HCS, but highly significant differences in cBMD between the female ICA and female HCS groups ( $p < 0.000$ ).

### Conclusions

Age of menarche and secondary amenorrhea are significantly associated with cBMD and history of BSI in HCS subjects, respectively. Differences in cBMD among the HCS subjects

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were not related to activity level. cBMD was significantly lower in female HCS as compared to female ICA. This difference in cBMD between ICA and HCS may be activity related.

## Level of Evidence

Level 3

## INTRODUCTION

Bone health is a significant global health issue facing our 21<sup>st</sup> century world. In 2000, there was estimated to be nine million osteoporotic fractures across the world, with hip, forearm, and vertebral fractures among the most common.<sup>1</sup> Such fractures lead to decreased functional ability, inability to fulfill social roles, loss of independence, and higher mortality.<sup>2</sup> The personal, social, and economic burden of poor bone health requires attention to this issue and close examination of the factors leading to poor bone health with aging.

Multiple factors have an influence on bone health including genetics, nutrition, physical activity, sex, age, and ethnicity. Some of those factors are non-modifiable (age, sex, genetics) while others including nutrition and physical activity are modifiable. The majority of bone mass is achieved by late adolescence, and, as such, maximizing bone accrual during adolescence is crucial in the prevention of early bone demineralization.<sup>3</sup> Given that premenopausal bone fractures have been shown to increase the risk for future fractures in a woman's life,<sup>4,5</sup> attention to bone health in young women is critical. Bone mineral density (BMD) is one important indicator of bone health, and several studies have demonstrated a positive relationship between increased physical activity and higher BMD.<sup>6-12</sup> A common theme to these studies is the importance of ground based, moderate intensity exercise as a stimulus for higher BMD. Studies by Reinking et al<sup>13</sup> and Risser et al<sup>14</sup> of BMD in collegiate athletes have shown lower BMD in swimmers as compared to ground-based athletes.

Given this evidence, it would be anticipated that participation in weight bearing sports would result in increased BMD. However, some evidence has shown lower BMD in endurance athletes as compared to a group of active, non-athletic subjects.<sup>15-19</sup> Bone stress injury, including stress fracture and medial tibial stress syndrome, are commonly experienced in running and jumping athletes.<sup>16-24</sup> Consequently, the dose-response relationship between weight-bearing physical activity and BMD is not fully understood. To encourage optimal bone health in a young adult population and minimize risk of bone demineralization and fracture in older adults, a deeper understanding of this relationship is critical.

The calcaneus is the only site recognized by the International Society of Clinical Densitometry (ISCD) for the assessment of bone density using quantitative ultrasound (QUS) bone densitometry.<sup>25</sup> Consisting of 75-95% trabecular bone, the calcaneus demonstrates greater degradation in response to age and disease compared to cortical bone due to its high rate of turnover, and structurally is well-suited for the horizontal transmission of sound energy due to its two relatively opposed lateral surfaces and minimal amount of soft tissue. The speed of sound (SOS) is deter-

mined by measuring the time it takes for sound energy to travel across the width of the calcaneus, and as such, is measured in units of meters per second (m/s). Broadband ultrasound attenuation (BUA) is the slope of the line formed by plotting sound intensity (dB) across a range of sound frequencies (MHz) and is reported in units of dB/MHz. BUA has been reported to provide a general indicator of bone quality, or architecture, rather than the quantity of mineral in bone.<sup>26</sup>

These measures collectively provide qualitative and quantitative information regarding the density, microarchitecture, and mechanical properties of the calcaneus. Faster SOS and greater BUA reflect more dense and homogenous bone structure, respectively, and when combined linearly provide a measure of relative bone "stiffness" referred to as the quantitative ultrasound index (QUI). The QUI value is rescaled to provide an estimate of BMD (g/cm<sup>2</sup>) allowing for determination of a t-score and comparison to previously established normative data. *In vivo* and *in vitro* QUS measures have been shown to be highly correlated ( $r = 0.82 - 0.85$ ) with other measures of calcaneal bone quality such as dual-energy x-ray absorptiometry (DXA), and with measures of bone density obtained at other anatomical locations such as the femur and lumbar spine.<sup>27-32</sup>

At present, inconsistent evidence is available pertaining to BMD values in athletic and non-athletic collegiate-aged persons, and the influence of physical activity on those values. Based on the literature review and the authors' previous work with cBMD in intercollegiate athletes, we established three purposes of this study: 1) compare cBMD of intercollegiate athlete (ICA) and healthy college students (HCS), 2) examine the influence of physical activity on cBMD in HCS, 3) investigate the effects of multiple variables on cBMD including body mass index (BMI), sex, menstrual history in females, and history of bone stress injury (BSI). Previously published data on ICAs were used to compare cBMD values obtained in HCS subjects.<sup>13</sup>

## METHODS

### STUDY DESIGN

Following approval by the Saint Louis University Institutional Review Board, a cross-sectional sample of HCS was recruited to participate in this study. Subjects who elected to participate in the research study provided consent and were scheduled for data collection. Data collection consisted of subjects completing a health and activity questionnaire and members of the research team collecting calcaneal bone mineral density, height, and weight measures.

### SUBJECTS

Full-time college students (82 female, 21 male) at one academic institution between 18-24 years of age ( $21.9 \pm 1.13$ )

who were not currently or previously rostered as a member of an intercollegiate athletic team were recruited to participate. Students not meeting the inclusion criteria or who described an existing lower-extremity injury were excluded from the study.

## PROCEDURES

### HEALTH & ACTIVITY QUESTIONNAIRE

Participants completed a web-based questionnaire including date of birth, sex, age, race/ethnicity, use of foot orthotics, lifetime history of diagnosed lower extremity or pelvic stress fracture or medial tibial stress syndrome (MTSS), and menstrual history. The Habitual Physical Activity Questionnaire was included in the web-based questionnaire as a measure of self-reported activity level.<sup>33</sup> The Habitual Physical Activity Questionnaire assesses activity across work, sport, and leisure not involving sport, and has been found to be a valid measure of activity level. The questionnaire consists of 16 items scored on a five-point scale which are used to calculate composite and categorical measures of self-reported physical activity.

### HEIGHT AND WEIGHT

Height (inches) and weight (pounds) were recorded using conventional methods employing a standard scale and tape measure. Height and weight measures were converted to the metric scale to allow for calculation of body mass index ( $\text{kg}/\text{m}^2$ ).

### CALCANEAL DENSITOMETRY

Quantitative ultrasound (QUS) involves the measurement of speed of sound (SOS) and attenuation of sound energy, or broadband ultrasound attenuation (BUA), as the sound wave passes through soft tissue and bone. Based on SOS and BUA measures, the quantitative ultrasound index (QUI) is obtained through simple linear combination of both values and then rescaled to give an estimate of bone mineral density. For the current study, calcaneal bone mineral density (cBMD) was determined via QUS using the Sahara clinical bone sonometer (Hologic, Inc, Waltham, MA). The Sahara clinical bone sonometer uses a specific technique involving the transmission of sound energy from one transducer through the calcaneus to a second receiver.<sup>31,34,35</sup>

Calibration of the densitometer was performed as described by manufacturer guidelines each day prior to data collection. Participants were seated in a straight-back chair approximately 12-18 inches from the scanner and both heels were inspected for abrasions or open sores. The tester cleaned the sides of the heels with a towelette and dried the heel prior to testing. The tester then applied an oil-based coupling gel (Hologic, Inc.) to both elastomer transducer pads. The subject's foot was then placed into the foot well and the leg secured using the positioning aid ([Figure 1](#)). The subject was instructed to remain still during the test and heel placement was checked to ensure proper placement prior to initiating the measurement. Once initiated, the elastomer pads moved to the measurement position contacting the sides of the calcaneus and measurement



**Figure 1. Measurement of Calcaneal Bone Mineral Density (cBMD)**

was performed. Upon completion of the measurement, SOS ( $\text{m}/\text{s}$ ), BUA ( $\text{dB}/\text{MHz}$ ), estimated cBMD ( $\text{g}/\text{cm}^2$ ), and BMD t-scores were recorded, and the steps repeated for the subject's contralateral calcaneus.

## DATA ANALYSIS

Descriptive statistics (means, percentages) for height, weight, and BMI were calculated using the demographic data of HCS subjects. The independent group t-test was used to compare self-reported physical activity scores to QUS measures in the group of HCS subjects. Self-reported physical activity, BMI, sex, and menstrual function were compared between HCS subjects with a history of BSI to the HCS subjects who denied a history of BSI using analyses of relationships (chi-square and relative risk). Comparison was made between QUS data collected on the group of 84 ICAs<sup>13</sup> (64 female, 20 male) to the QUS data from the 103 HCS in this study using the independent group t-test. R statistical computing environment (R Core Team, 2013) was used for data management and analysis.

## RELIABILITY

Reliability of the calcaneal bone densitometer (cBMD, SOS) was determined through random selection of subjects to have repeated density measures of both calcanei at the time of initial data collection. Intrarater reliability, calculated using intraclass correlation coefficients (ICC 3.1) for cBMD, were previously reported as demonstrating a high level of measurement consistency ( $> 0.95$ ).<sup>13</sup>

## RESULTS

Participant demographics for the HCS subjects are summarized in [Table 1](#) and self-reported history of stress fracture or leg pain (defined as pain occurring on the inside of the lower leg between the knee and ankle) is reported in [Table 2](#).

**Table 1. Healthy College Student Demographics**

	Healthy College Students (n=103)*	
	Female (n=82)	Male (n=21)
Height (m)	1.64 (0.06)	1.78 (0.07)
Weight (kg)	63.26 (8.65) <sup>a</sup>	85.57 (15.95) <sup>a</sup>
BMI (kg/m <sup>2</sup> )	23.46 (2.95) <sup>b</sup>	26.93 (3.92) <sup>b</sup>

BMI = body mass index

\*Values are mean ± SD

<sup>a,b</sup>Significantly different at  $p < 0.001$ **Table 2. Healthy College Student Bone Stress Injury History**

	Healthy College Students (%)	
	Total (n=103)	Female (n=82)
Stress Fracture Hx	8 (7.8)	8 (9.8)
Leg Pain Hx	29 (28.2)	25 (30.5)

Hx = history

Twenty HCS (19.4%) reported participating in organized sports and 48 HCS (46.6%) reported “more” or “much more” physical activity compared to their peers on the Habitual Physical Activity Questionnaire. Habitual Physical Activity Scores were not associated with self-reported history of BSI (stress fracture or MTSS) or with cBMD or SOS measures. However, a small but significant negative association between BMI and the Habitual Physical Activity work subscale was identified across all HCS subjects ( $r = -0.29$ ,  $p < 0.05$ ) and in the female HCS group ( $r = -0.23$ ,  $p < 0.05$ ). Small but significant negative associations were also found in the female HCS group between age of menarche and cBMD in both the left ( $r = -0.22$ ,  $p < 0.05$ ) and right ( $r = -0.23$ ,  $p < 0.05$ ) calcanei, and between a history of stress fracture in the pelvis or lower extremity and a history of secondary amenorrhea ( $r = 0.32$ ,  $p < 0.05$ , [Table 3](#)).

Calcaneal QUS measures (cBMD, SOS) for all HCS subjects were significantly lower ( $p < 0.000$ ) than preseason measures obtained in ICAs ([Table 4](#)). Calcaneal BMD and SOS measures were significantly lower in HCS females compared to ICA females; however, male ICA and HCS had cBMD and SOS measures that were not significantly different ([Table 4](#)). From the cBMD reliability study the intraclass correlation coefficient values (ICC 3,1) were all greater than 0.95, indicating a high level of measurement consistency.

## DISCUSSION

### PHYSICAL ACTIVITY AND CBMD IN HCS

Although numerous studies have reported the benefits of exercise and sport on bone accrual and bone strength, few studies have examined the relationship between physical activity level and optimal bone health.<sup>36–38</sup> While athletes have been shown to have greater BMD and bone health when compared to a referent group of non-athletes, the

level of physical activity necessary to stimulate an osteogenic response has not been identified. Researchers have provided conflicting evidence to support the benefits of physical activity on bone health in the general population.<sup>6,36,38–40</sup> Thus, the second purpose of the study was to examine the relationship between self-reported physical activity and cBMD in a group of HCS. The results did not identify a significant relationship between self-reported physical activity as measured by the Habitual Physical Activity Questionnaire and cBMD or SOS in this group of HCS. Comparing the results to previous studies is difficult due to differences in the methods used to assess physical activity level. It is also important to note the limitations of available methods used to measure physical activity. For example, the Habitual Physical Activity Questionnaire addresses frequency of exercise but may not account for osteogenic differences between various exercise modes. Therefore, subjects reporting higher levels of physical activity who are performing activities that are generally considered low-load, endurance activities are less likely to demonstrate significant differences in bone development and structure when compared to less physically active subjects. Wallace et al<sup>38</sup> assessed both historical and current physical activity level in their study and reported that lifetime physical activity and lifetime weight-bearing activity accounted for 3–15% of the variance in BMD in a group of 19–25 year old regularly menstruating females. Sawyer et al<sup>39</sup> used calcaneal QUS in a group of healthy subjects aged 6.6–20 years of age ( $n=311$ ) and found a significant relationship between BUA and SOS and physical activity. However, when accounting for age and weight, physical activity accounted for only 1.0–1.4% of the variance in SOS and bone stiffness. In a study of 60 healthy, eumenorrheic women aged 25–34 years of age, physical activity was found to be significantly associated with vertebral bone density ( $r = 0.41$ ,  $p < 0.005$ ).<sup>36</sup> A study of adolescent females with a history of an eating dis-

order were compared to matched females with no history of eating disorder who were also grouped based on performing more or less than seven hours of physical activity per week. No differences in total body or site-specific BMD were found between the eating disorder group or two activity groups.<sup>40</sup>

#### ASSOCIATION BETWEEN BONE STRESS INJURY AND CBMD, BMI, SEX, AND MENSTRUAL FUNCTION

Bone stress injuries have been identified as a common injury among endurance athletes. The long-term consequences of maladaptive changes in bone microarchitecture and bone geometry to repetitive loading have not been studied. Methods to assess bone health during periods of development, changes in activity, and with ageing will allow medical professionals to identify risk factors for bone stress injury and evaluate interventions designed to influence bone health. Quantitative ultrasound has also been shown to have similar prediction capability for fracture risk as compared to DXA and has been shown to correlate with DXA measures in young children and adolescents.<sup>31,34,35</sup> Van Mechelen<sup>41</sup> has described a “sequence” for the prevention of sports injury which includes identifying factors which play a role in the occurrence of sports injury followed by the introduction of measures to address the aforementioned factors.

A number of authors have shown significantly altered bone microarchitecture and lower bone density and bone strength in athletes with stress fracture or bone stress injury when compared to healthy athletes.<sup>21,42–44</sup> The authors of this study have shown that QUS measures in female athletes involved in ground based sports who develop a bone stress injury during the competitive season are significantly lower relative to their peers.<sup>13</sup> It is worth noting that studies that did not find a significant difference in BMD between subjects with bone stress injury and controls reported important methodological differences. For example, Bennell et al<sup>45</sup> reported no significant difference in BMD when comparing female runners with a history of healed tibial stress fracture to female runners without a history of stress fracture, suggesting that timing of the densitometry measurement relative to the diagnosis of bone stress injury may influence BMD measures. In a study comparing military recruits who developed a bone stress injury to healthy, matched controls, there were no differences in BMD measures at the tibia and femoral neck.<sup>46</sup> However, military recruits present with diverse levels of physical activity history, meaning the change in volume of physical activity likely varied across subjects with basic training and therefore exposed the subjects to varied levels of risk.

A number of authors have examined the influence of menstruation, BMI, and sex on risk of developing bone stress injury in athletes and the military population. When comparing cBMD between groups and blocking for sex, ICA male and HCS male subjects were found to have similar cBMD and SOS while female subjects had significantly different cBMD and SOS measures across ICA and HCS groups. This finding suggests the influence of sex linked factors in bone accrual and structure and supports previous studies demonstrating differences between male and female bone geometry and strength.<sup>35,47</sup> For example, Beck et al<sup>47</sup> found

significantly smaller subperiosteal diameters and thinner cortices in female military recruits compared to male recruits. Several authors reporting on the incidence of stress fracture in military and athletic populations have identified female sex as a risk factor for bone stress injury.<sup>47–49</sup>

The influence of menstruation on bone health and in the development of bone stress injury has been described extensively as part of the female athlete triad, and the results of this study support the relationship between menstrual abnormality and low bone density.<sup>50–55</sup> More specifically, amenorrhea has been shown to be a significant risk factor for bone stress injury in female athletes. The current study demonstrated a small but significant negative correlation ( $r = -0.22$  and  $r = -0.23$ ) between age of menarche and cBMD, suggesting an important relationship between earlier onset of menstruation and greater bone accrual during adolescence. A history of amenorrhea was also found to be associated with a history of stress fracture in the pelvis or lower extremity, supporting previous studies that have shown a relationship between menstruation and risk of bone stress injury. While differences in BMI across sports are common, the authors did not identify a relationship between BMI and cBMD in either the ICA or HCS groups.

#### CBMD IN ICA VS HCS

This is the first known study to compare measures of bone health (SOS & BUA) in ICA and HCS using calcaneal QUS bone densitometry. The authors found significant differences in QUS measures for cBMD ( $p < 0.000$ ) and SOS ( $p < 0.000$ ) between the female ICA and female HCS groups, with female ICA having significantly greater cBMD. Previous studies have described the anabolic effects of physical activity on bone structure, especially in athletes involved in high-impact sports and weight training.<sup>7,11,12</sup> Tenforde et al<sup>12</sup> in their review of the literature found an association between impact sports (gymnastics, soccer, etc.) and greater bone mineral density, bone composition and bone geometry, while non-impact sports such as cycling and water polo were not associated with increased bone quality or quantity, and in the case of swimming were negatively associated. The authors of this manuscript reported similar findings in a sample of ICA, with significantly lower cBMD and SOS in swimmers & divers when compared to soccer and cross-country/track athletes.<sup>13</sup> Considering ICA are typically engaged in high load activities associated with their sport and participate in regular strengthening and conditioning programs, it is not surprising that we found significantly higher measures of bone quality and quantity when compared to HCS subjects. However, when the QUS measures in ICA who developed BSI during their competitive season were compared to the QUS measures in HCS, there was no significant difference in cBMD or SOS between groups. This study consisted of a sample of convenience of healthy college students at one Midwest institution with a greater number of female than male subjects and therefore generalizability is limited. History of BSI relied on self-reporting and therefore is dependent on subject recall and susceptible to bias.

**Table 3. Association Between Variables in Female Healthy College Students**

	RcBMD	RSOS	LcBMD	LSOS	BMI	FXDX	MENA	AMEN2 HX	OLIGO HX	AMEN2 YR	OLIGO YR
RcBMD	1.00										
RSOS	.96 <sup>a</sup>	1.00									
LcBMD	.88 <sup>b</sup>	.89 <sup>b</sup>	1.00								
LSOS	.89 <sup>c</sup>	.93 <sup>c</sup>	.97 <sup>c</sup>	1.00							
BMI	-.04	-.09	-.05	-.13	1.00						
FXDX	-.03	-.05	-.11	-.12	.27 <sup>d</sup>	1.00					
MENA	-.23 <sup>e</sup>	-.18	-.22 <sup>e</sup>	-.17	-.19	.08	1.00				
AMEN2 HX	-.07	-.07	-.02	-.07	.12	.32 <sup>f</sup>	.18	1.00			
OLIGO HX	.01	.01	.00	.01	-.01	.10	.19	.32 <sup>g</sup>	1.00		
AMEN2 YR	-.12	-.11	.02	-.03	-.04	-.09	-.03	.42 <sup>h</sup>	.17	1.00	
OLIGO YR	.18	.18	.15	.15	-.02	-.10	-.13	.15	.54 <sup>i</sup>	.08	1.00

RcBMD = right calcaneal bone mineral density; RSOS = right speed of sound; LcBMD = left calcaneal bone mineral density; LSOS = left speed of sound; BMI = body mass index; FXDX= stress fracture diagnosis; MENA = age of menarche; AMEN2 HX = secondary amenorrhea history not including the last 12 months; OLIGO HX = oligomenorrhea history not including the last 12 months; AMEN2 YR = secondary amenorrhea in the last 12 months; OLIGO YR = oligomenorrhea in the last 12 months

a,b,c,h,i Association between variables significant at  $p < 0.001$

d,e,f,g Association between variables significant at  $p < 0.05$



**Table 4. Speed of Sound and Calcaneal Bone Mineral Density Quantitative Ultrasound Measures Between Intercollegiate Athlete and Healthy College Students**

	Quantitative Ultrasound Measures*			
	RSOS (m/s)	LSOS (m/s)	RcBMD (g/cm <sup>2</sup> )	LcBMD (g/cm <sup>2</sup> )
ICA (n=84)	1605.2 ± 36.1 <sup>a</sup>	1604.3 ± 33.4 <sup>b</sup>	.710 ± .15 <sup>c</sup>	.698 ± .14 <sup>d</sup>
HCS (n=103)	1582.0 ± 32.3 <sup>a</sup>	1582.0 ± 34.1 <sup>b</sup>	.615 ± .13 <sup>c</sup>	.617 ± .14 <sup>d</sup>
Male ICA (n=20)	1597.1 ± 33.5	1595.9 ± 33.4	.687 ± .13	.681 ± .13
Male HCS (n=21)	1601.7 ± 33.1	1597.0 ± 34.8	.693 ± .13	.688 ± .15
Female ICA (n=64)	1607.8 ± 36.8 <sup>e</sup>	1606.9 ± 34.2 <sup>f</sup>	.717 ± .16 <sup>g</sup>	.703 ± .14 <sup>h</sup>
Female HCS (n=82)	1577.0 ± 30.3 <sup>e</sup>	1578.1 ± 33.0 <sup>f</sup>	.596 ± .12 <sup>g</sup>	.599 ± .13 <sup>h</sup>

RSOS = right speed of sound; LSOS = left speed of sound; RcBMD = right calcaneal bone mineral density; LcBMD = left calcaneal bone mineral density; ICA = intercollegiate athlete; HCS = healthy college students

\*Values are mean ± SD

<sup>a-h</sup>p < 0.000

## CONCLUSION

Methods to assess bone health are valuable to medical professionals involved in the prevention and treatment of bone stress injuries. A thorough understanding of the influence of physical activity, menstrual history, sex, BMI, BMD, and history of BSI on bone health will aide in the management of subjects at risk of or diagnosed with a bone stress injury. Age of menarche and history of amenorrhea were found to be significantly associated with cBMD and self-reported stress fracture history, respectively, and should be considered when assessing the risk of bone stress injury in females. Female HCS with no history of intercollegiate athletic participation were found to have significantly lower cBMD than a group of female ICA. The results of this study suggest that QUS is a valuable instrument for identifying subjects with low calcaneal bone mineral density who may be at risk of developing a BSI. As a non-ionizing, efficient, portable, and low-cost modality, QUS should be considered

a viable instrument in monitoring the response of bone to repetitive stress, identifying athletes with low BMD at increased risk of bone stress injury, and prescribing optimal osteogenic interventions in the management of bone stress injury. Future studies should include a larger sample of collegiate and non-collegiate athletes and non-athletes and include additional risk factors that will further help identify level of risk of bone stress injury.

## CONFLICTS OF INTEREST

The authors have no conflicts to disclose

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